ELECTRON DONORS FOR CHLORINATED SOLVENT SOURCE AREA BIOREMEDIATION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/404,728, filed August 20, 2002.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not applicable.

BACKGROUND OF THE INVENTION

This invention relates to remediation of environmental contamination. More particularly, the invention relates to methods for accelerating or enhancing *in situ* dehalogenation of nonaqueous halogenated solvents in ground water. These methods involve adding to the contaminated ground water a composition of matter that both functions as an electron donor for halorespiration processes carried out by indigenous or exogenously supplied bacteria, wherein the nonaqueous halogenated solvents are dehalogenated and degraded to innocuous compounds, and promotes mass transfer of the nonaqueous halogenated solvents from a source into the ground water where such solvents can be broken down.

For many years little care was taken in the handling of organic solvents and other materials that were used in industry and at government installations, such as military bases. Because of poor handling techniques and, occasionally, intentional dumping, many industrial

sites and military bases now have contaminated areas containing relatively high concentrations of these contaminants. Chlorinated solvents, such as trichloroethylene (TCE), perchloroethylene (PCE), and other types of liquids, are common at such sites, and if not removed can infiltrate groundwater supplies, rendering the water unfit for consumption and other uses.

A variety of techniques have been used to promote the removal of such chemical contaminants, both from the soil and from the ground water. The principle method of ground water remediation currently used where dense, non-aqueous phase liquids (DNAPLs) are involved is what is commonly referred to as "pump-and-treat" remediation. According to this method, wells are drilled into the contaminated area and contaminated ground water is pumped above the surface, where it is treated to remove the contaminants.

The limitations of the pump-and-treat method have been documented in articles such as D.M. Mackay & J.A. Cherry, Groundwater Contamination: Pump and Treat Remediation, 23 Environ. Sci. Technol. 630-636 (1989). The authors of this article concluded that pump-and-treat remediation can only be relied on to contain ground water contamination through the manipulation of hydraulic gradients within an aquifer. The reasons for the failure of the pump-and-treat method to decontaminate aquifers are rooted in the limited aqueous solubility of many DNAPLs in ground water and other processes involving contaminant desorption and diffusion. Because of the low aqueous solubility of most DNAPLs, their removal by ground water extraction requires exceptionally long periods of time.

Due to the general impracticability of the pump-and-treat method, considerable attention has been paid recently to other methods for effecting remediation. One such process is commonly referred to as enhanced solubilization. This method uses micellar surfactants to

increase the effective solubility of the DNAPLs to accelerate the rate of removal. The mechanism of solubilization displayed by surfactants arises from the formation of microemulsions by the surfactants, water, and the solubilized DNAPLs. For example, Table 1 shows solubilization of PCE by various nonionic and anionic surfactants. These data indicate that even dilute surfactants can significantly increase the aqueous solubility of PCE.

Table 1

Surfactant	Surfactant Concentration	PCE Solubilized (mg/l)
Water	0%	240
Nonylphenol ethoxylate and its phosphate ester (1:1)	2%	11,700
Sodium diamyl and dioctyl sulfosuccinates (1:1) in 500 mg $CaCl_2$/l	4%	85,000
Nonylphenol ethoxylate	1%	1,300

A serious drawback with the surfactant-enhanced aquifer remediation is that the vertical mobility of the solubilized DNAPLs substantially requires that an aquiclude be present to catch any solubilized contaminant that migrates vertically. Many aquifers, however, lack such an aquiclude. If the traditional surfactant-enhanced aquifer remediation method were to be used with an aquifer lacking an aquiclude, there is a significant risk that the solubilized DNAPLs will spread vertically and contaminate an increasingly large volume. Another drawback of surfactant-enhanced aquifer remediation is the need to pump high concentrations of contaminated water above ground, which results in exposure risks to workers and the environment, and the need to dispose or recycle the surfactant.

Another method for effecting remediation of ground water contaminated with DNAPLs is

known as enhanced bioremediation. Enhanced bioremediation, as opposed to intrinsic bioremediation, of halogenated solvent-contaminated ground water falls into the two broad categories of aerobic and anaerobic bioremediation. The aerobic processes, regardless of whether they are carried out *in situ* or in a bioreactor, require addition of (1) oxygen as the electron acceptor for catabolism of the halogenated solvents, and (2) a carbon source, such as methane, propane, phenol, toluene, or butane. The utilization of an appropriate carbon source induces an enzyme that fortuitously degrades many halogenated solvents, but without any immediate benefit to the microorganisms involved. This process has been applied *in situ* to aqueous contamination in several instances, and at least one patent has been granted for this approach (U.S. Patent No. 5,384,048). It has also been used to treat aqueous contamination in above-ground bioreactors with numerous variations, especially using proprietary microorganisms and nutrient mixes. Many patents have been granted in this area, e.g., U.S. Patent No. 5,057,221; U.S. Patent No. 5,962,305; U.S. Patent No. 5,945,331.

Anaerobic bioremediation of halogenated solvents is a fundamentally different process than aerobic bioremediation. Under appropriate anaerobic conditions, chlorinated solvents can be used directly by some microorganisms as electron acceptors through a process that has come to be known as "chlororespiration," or, more generally, "halorespiration." D.L. Freedman & J.M. Gossett, Biological Reductive Dechlorination of Tetrachloroethylene and Trichloroethylene to Ethylene Under Methanogenic Conditions, 55 Applied Environ. Microbiol. 2144-2155 (1989), first published the complete degradation pathway for chlorinated ethenes to ethene. In the following years, several publications reported evidence that the degradation could be achieved through microbial respiration, indicating that the microorganisms could actually grow by using

chlorinated solvents directly as electron acceptors. The primary requirement to facilitate this process is the addition of a suitable electron donor or carbon source. Many electron donors have been described in the literature, including acetate, lactate, propionate, butyrate, formate, ethanol, hydrogen, and many others. U.S. Patent No. 5,277,815 issued in 1994 for *in situ* electron donor addition along with control of redox conditions to effect the desired end products. U.S. Patent No. 5,578,210 issued later for enhanced anaerobic *in situ* bioremediation using "biotransformation enhancing agents," i.e., electron donors such as propylene glycol, glycerol, glutamate, a mixture of proteose peptone, beef extract, yeast extract, malt extract, dextrose, and ascorbic acid, and mixtures thereof. Based primarily on what was publicly available in the scientific literature, studies of enhanced anaerobic *in situ* bioremediation of chlorinated solvents began in the mid-1990s. This approach generally includes electron donor addition, sometimes with other micronutrients, to facilitate biotransformation of aqueous-phase contaminants. To date, only a few large-scale studies have been published in the peer-reviewed literature, but environmental consulting companies and remediation contractors are increasingly using the general approach.

With one very recent exception, discussed below, all of the work done in this area to date has focused on the biodegradation of aqueous contaminants, because microorganisms cannot directly degrade nonaqueous contaminants. Consequently, bioremediation is not generally thought to be applicable to sites with residual DNAPLs in the subsurface. Therefore, the technologies currently in use include thermal technologies such as steam stripping, *in situ* chemical oxidation, surfactant flushing, or co-solvent flushing. Surfactant (or co-solvent) flushing, briefly described above, is a chemical process that aims to facilitate transport of

nonaqueous contaminants, but without attention to biodegradation. At many sites, however, the pump-and-treat process continues to be used to hydraulically contain residual source areas although it is almost universally accepted that these systems will have to operate in perpetuity because of their inefficient removal of nonaqueous contaminants.

The notable recent exception to the focus of bioremediation on aqueous contaminants away from residual source areas is a study by C.S. Carr et al., Effect of Dechlorinating Bacteria on the Longevity and Composition of PCE-Containing Nonaqueous Phase Liquids under Equilibrium Dissolution Conditions, 34 Environ. Sci. Technol. 1088-1094 (2000), demonstrating that anaerobic bioremediation of tetrachloroethene (PCE) enhanced mass transfer from the nonaqueous phase to the aqueous phase and significantly shortened the longevity of the nonaqueous source. The mechanisms identified were (1) enhanced dissolution of PCE resulting from the continuous removal of the compound from the aqueous phase by bacteria, and (2) increased solubility of the intermediate chlorinated ethenes relative to PCE, allowing the total moles of chlorinated ethenes in the aqueous phase to increase due to biotransformation. This study is important because it identifies some of the advantages of enhancing mass transfer from the nonaqueous phase to the aqueous phase.

In view of the foregoing, it will be appreciated that providing methods for accelerating or enhancing *in situ* bioremediation of halogenated solvents in ground water would be a significant advancement in the art.

BRIEF SUMMARY OF THE INVENTION

It is an advantage of the present invention to provide a method for *in situ* remediation of

DNAPLs in ground water wherein capital costs are low.

It is also an advantage of the invention to provide a method for *in situ* remediation of DNAPLs in ground water wherein mass transfer from the nonaqueous phase to the aqueous phase is enhanced.

It is another advantage of the invention to provide a method for *in situ* remediation of DNAPLs in ground water wherein the longevity of source areas is shortened.

It is still another advantage of the invention to provide a method for *in situ* remediation of DNAPLs in ground water wherein no extraction of contaminated water from the ground is required.

It is yet another advantage of the invention to provide a method for *in situ* remediation of DNAPLs in ground water such that the concentrations of the solvents are restored to below regulatory limits and no follow-on remediation activities, other than perhaps monitored natural attenuation, are needed.

It is a still further advantage of the invention to provide a method for *in situ* remediation of DNAPLs in ground water wherein the DNAPLs are more rapidly removed from the ground water than with prior art methods and residual source areas are removed.

It is another advantage of the invention to provide a method for *in situ* remediation of DNAPLs in ground water wherein transport is facilitated and bioavailability of nonaqueous halogenated solvents is enhanced.

It is still another advantage of the invention to provide a method for *in situ* remediation of DNAPLs in ground water wherein the method is sustainable for low cost and has low maintenance requirements.

It is yet another advantage of the invention to provide a method for *in situ* remediation of DNAPLs in ground water by adding a composition of matter that is both an electron donor and a surfactant or enhancer of mass transfer.

It is still further an advantage of the invention to provide a method for remediation of DNAPLs in ground water wherein destruction of the DNAPLs occurs *in situ*.

It is a yet further advantage of the invention to provide a method for *in situ* remediation of DNAPLs in ground water wherein an unobtrusive appearance is provided and it meets with public acceptance.

These and other advantages can be addressed by providing a method for enhancing *in situ* bioremediation of a nonaqueous halogenated solvent in ground water comprising adding to the ground water an amount of an electron donor sufficient for a halo-respiring microbe in the ground water to use the nonaqueous halogenated solvent as an electron acceptor, thereby reductively dehalogenating the nonaqueous halogenated solvent into innocuous compounds, wherein the electron donor enhances mass transfer of the nonaqueous halogenated solvents into solution. The electron donor illustratively functions as a surfactant or co-solvent. In cases where the electron donor is a functional surfactant, it is illustratively added at a concentration above the critical micelle concentration in water. In cases where the electron donor is a functional co-solvent, there may be no critical micelle concentration, or if there is a critical micelle concentration in water, the electron donor is illustratively added at a concentration below such critical micelle concentration. Illustrative electron donors for use in this method include C_2-C_4 carboxylic acids and hydroxy acids, salts thereof, esters of C_2-C_4 carboxylic acids and hydroxy acids, and mixtures thereof. Other illustrative electron donors according to the present invention

include oleyl lactylic acid, linoleyl lactylic acid, linolenoyl lacylic acid, stearoyl lactylic acid, palmitoyl lactylic acid, myristoyl lactylic acid, lauroyl lactylic acid, caproyl lactylic acid, mixtures thereof, mixtures with fatty acids or salts thereof, mixtures with lactic acid or salts thereof, mixtures with fatty acids and lactic acid and salts thereof, and the like. In a specific embodiment of the invention, the electron donor is a member selected from the group consisting of lactic acid, salts thereof, lactate esters, and mixtures thereof. Illustrative salts of lactic acid include sodium lactate, potassium lactate, lithium lactate, ammonium lactate, calcium lactate, magnesium lactate, manganese lactate, zinc lactate, ferrous lactate, aluminum lactate, and mixtures thereof, wherein sodium lactate is especially illustrative. In another specific embodiment of the invention, the electron donor is a member selected from the group consisting of oleyl lactylic acid, oleic acid or salts thereof, and lactic acid or salts thereof. Illustrative targets of the method include nonaqueous chlorinated solvents, such as perchloroethylene (PCE), trichloroethylene (TCE), dichloroethylene (DCE), vinyl chloride (VC), 1,1,1-trichloroethane (TCA), carbon tetrachloride and less chlorinated derivatives thereof, and mixtures thereof. A specific aspect of the invention relates to enhancing the reductive dehalogenation activity of indigenous halo-respiring microbes present in the ground water. If halo-respiring microbes are absent or ineffective, then such microbes can be exogenously supplied to the ground water. Illustratively, the microbes are bacteria, such as *Dehalococcoides ethenogenes* strain 195, the Pinellas culture, and the like, and mixtures thereof. The method degrades the halogenated solvents into innocuous compounds such as ethylene, ethane, carbon dioxide, water, halogen salts, and mixtures thereof.

A method for enhancing mass transfer of a nonaqueous halogenated solvent present in a

nonaqueous residual source of contamination into the aqueous phase comprises adding to the ground water an effective amount of a composition that donates electrons for reductive dehalogenation of the nonaqueous halogenated solvent and functions as a surfactant for solubilizing the nonaqueous halogenated solvent.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

FIG. 1 is a site plan of Test Area North showing the locations of injection wells (○) and monitoring well (●).

FIG. 2 is a cross section of Test Area North showing the locations and relative depths of injections wells (open bars), monitoring wells (closed bars), and open or screened intervals (hatched bars).

FIGS. 3A-C show the relationship of electron donor concentrations and redox conditions to reductive dechlorination at well TAN-31. FIG. 3A shows COD (solid line) and electron donor (broken line) concentrations in units of mg/L as a function of time in days. FIG. 3B shows ferrous iron (dotted line), sulfate (solid line), and methane (dashed line) concentrations in units of mg/L as a function of time in days. FIG. 3C shows TCE, *cis*-DCE, *trans*-DCE, VC, and ethene concentrations in units of μmol/L as a function of time in days.

FIG. 4 shows facilitated TCE transport and subsequent biodegradation in well TAN-26.

FIG. 5 shows surface tension as a function of lactate concentration for sodium lactate solutions without added ethyl lactate (♦) and with 0.1% ethyl lactate (X), 1% ethyl lactate (◊), and 10% ethyl lactate (*); error bars represent two standard deviations around the mean.

FIG. 6 shows interfacial tension as a function of lactate concentration for sodium lactate

solutions without added ethyl lactate (♦) and with 0.1% ethyl lactate (X), 1% ethyl lactate (◊), and 10% ethyl lactate (*); error bars represent two standard deviations around the mean.

FIG. 7 shows bioavailability enhancement followed by complete reductive dechlorination of TCE; symbols show injections of: □ - TCE; ▵ - cis-DCE; X - trans-DCE; ■ - VC; ● - ethene; ♦ - lactate.

FIG. 8 shows the impact of various electron donor solution concentrations on interfacial tension with TCE: ♦ - lactate; ▲ - whey powder; X - molasses; ● - oleyl lactyllic acid.

DETAILED DESCRIPTION

Before the present methods for accelerating or enhancing *in situ* bioremediation of halogenated solvents in ground water are disclosed and described, it is to be understood that this invention is not limited to the particular configurations, process steps, and materials disclosed herein as such configurations, process steps, and materials may vary somewhat. It is also to be understood that the terminology employed herein is used for the purpose of describing particular embodiments only and is not intended to be limiting since the scope of the present invention will be limited only by the appended claims and equivalents thereof.

The publications and other reference materials referred to herein to describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference. The references discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention.

It must be noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an electron donor" includes reference to a mixture of two or more of such electron donors, reference to "a solvent" includes reference to one or more of such solvents, and reference to "a microbe" includes reference to a mixture of two or more of such microbes.

In describing and claiming the present invention, the following terminology will be used in accordance with the definitions set out below.

As used herein, "comprising," "including," "containing," "characterized by," and grammatical equivalents thereof are inclusive or open-ended terms that do not exclude additional, unrecited elements or method steps. "Comprising" is to be interpreted as including the more restrictive terms "consisting of" and "consisting essentially of."

As used herein, "consisting of" and grammatical equivalents thereof exclude any element, step, or ingredient not specified in the claim.

As used herein, "consisting essentially of" and grammatical equivalents thereof limit the scope of a claim to the specified materials or steps and those that do not materially affect the basic and novel characteristic or characteristics of the claimed invention.

As used herein, "PCE," "perchloroethylene," "tetrachloroethylene," and "tetrachloroethene" refer to $Cl_2C=CCl_2$.

As used herein, "TCE," "trichloroethylene," and "trichloroethene" refer to $Cl_2C=CH–Cl$.

As used herein, "DCE," "dichloroethylene," and "dichloroethene" refer to Cl–HC=CH–Cl.

As used herein, "VC" and "vinyl chloride" refer to $H_2C=CH-Cl$.

As used herein, "ethylene" and "ethene" refer to $H_2C=CH_2$.

As used herein, "chloroethenes" means PCE, TCE, DCE, VC, and mixtures thereof.

As used herein, "biotransformation" means a biological reduction in the number of halogen, e.g., chlorine, atoms covalently bound to an organic compound. For example, PCE can be biotransformed to TCE, which can be biotransformed to DCE, which can be biotransformed to vinyl chloride, which can be biotransformed to ethylene. If the rate of biotransformation is increased by adding an electron donor to the ground water, then the biotransformation is enhanced.

As used herein, "microbe" means a microscopic organism, such as bacteria, protozoa, and some fungi and algae. Bacteria are especially illustrative microbes according to the present invention. Biotransformation is enhanced, at least in part, by stimulating indigenous, naturally occurring microbes in the ground water. If indigenous, naturally occurring microbes are not present or are not sufficiently effective, then an appropriate microbe can be added to the ground water, as well as the electron donor of the present invention. The microbe can be added before, with, or after adding the electron donor to the ground water. Illustratively, the microbe is an anaerobic or facultatively anaerobic bacterium. Bacteria known to work within the current processes include *Dehalococcoides ethenogenes* strain 195 (X. Maymo-Gatell et al., Isolation of a Bacterium that Reductively Dechlorinates Tetrachloroethene to Ethene, 276 Science 1568-1571 (1997)), the Pinellas culture (M.R. Harkness et al., Use of Bioaugmentation To Stimulate Complete Reductive Dechlorination of Trichloroethene in Dover Soil Columns, 33 Environmental Sci. Technol. 1100-1109 (1999); D.E. Ellis et al., Bioaugmentation for

Accelerated In Situ Anaerobic Bioremediation, 34 Environmental Sci. Technol. 2254-2260 (2000)), and the like, and mixtures thereof. Other species, however, are known to function, and the present invention is not limited by the examples provided herein.

As used herein, "surfactant" means a substance that when dissolved in water or an aqueous solution reduces its surface tension or the interfacial tension between it and another liquid. Surfactants are characterized by a structural balance between one or more hydrophilic and hydrophobic groups. This amphiphilic nature causes them to be preferentially adsorbed at air-water, oil-water, and solid-water interfaces, forming oriented monolayers wherein the hydrophilic groups are in the aqueous phase and the hydrocarbon chains are pointed toward the air, in contact with the solid surfaces, or immersed in the oil phase. Surfactants are characterized by a critical micelle concentration (cmc), a concentration at which surfactant molecules begin to aggregate into micelles and above which more micelles are formed. Surfactants enhance solubility of nonpolar compounds in aqueous solutions by providing a microenvironment, i.e., the interior of micelles, where the nonpolar compounds can accumulate. In certain illustrative embodiments of the present invention, the electron donor is a surfactant.

As used herein, a "co-solvent" is a solvent present in a minor amount as compared to a solvent with which it is mixed. Co-solvents are like surfactants in that they decrease interfacial tension between two liquid phases, but they generally do not form micelles. Thus, co-solvents enhance solubility, but not to the extent of surfactants. In the context of *in situ* bioremediation, the rate of enhanced solubilization mediated by a co-solvent or co-solvents is less likely to overwhelm the rate of biotransformation. Thus, in certain illustrative embodiments of the invention, the electron donor is a co-solvent.

Chlorinated solvents represent two of the three most common ground water contaminants at hazardous waste sites in the United States, and with their degradation products they account for eight of the top 20. Unfortunately, chlorinated solvents are relatively recalcitrant compounds with low, but toxologically significant, solubilities in water. Historically, the conventional technology for ground water treatment has been pump-and-treat methodology. While the pump-and-treat approach can be useful for achieving hydraulic containment of a ground water contaminated with chlorinated solvents, it has very rarely been successful for restoration, largely because of the heterogeneity of the subsurface (i.e., preferential flow paths) and the presence of nonaqueous phase liquids. This has led to significant research in the last 10 years on *in situ* technologies for restoration of ground water contaminated with chlorinated solvents.

Residual chlorinated solvent source areas (where nonaqueous contaminants are present) in the subsurface are especially problematic because the combination of low contaminant solubilities and the lack of mixing in typical ground water flow makes them very long-lived (decades to centuries). As discussed above, the common perception that bioremediation cannot effect improvements to the slow mass transfer from the nonaqueous to the aqueous phase has limited its applications to aqueous-phase contaminated ground water plumes. Also mentioned above, the technology categories used for these areas other than pump-and-treat include thermal technologies such as stream-stripping, *in situ* chemical oxidation, surfactant flushing, or co-solvent flushing. While these approaches generally result in some rapid mass removal of contaminants and have worked to varying degrees, they all share a common disadvantage: they have a high capital cost in the early stages of remediation. In addition, all except chemical oxidation require extraction of contaminants from the ground with subsequent treatment. This

creates new exposure pathways and increases costs. Finally, these technologies rarely restore ground water to contaminant concentrations below regulatory limits, so follow-on activities are generally required.

P.V. Roberts et al., Field Study of Organic Water Quality Changes during Ground Water Recharge in the Palo Alto Baylands, 16 Water Resources Research 1025-1035 (1982), reported one of the first field observations suggesting bioremediation of chloroethenes (PCE, TCE, DCE, and VC). E.J. Bouwer & P.L. McCarty, Transformation of 1- and 2-Carbon Halogenated Aliphatic Organic Compounds under Methanogenic Conditions, 45 Applied Environ. Microbiol. 1286-1294 (1983), confirmed biodegradation of PCE and TCE in the laboratory shortly thereafter. F. Parsons et al., Transformations of Tetrachloroethylene and Trichloroethylene in Microcosms and Groundwater, 76 J. Am. Water Works Ass'n 56-59 (1984), and T.M. Vogel & P.L. McCarty, Biotransformation of Tetrachloroethylene to Trichloroethylene, Dichloroethylene, Vinyl Chloride, and Carbon Dioxide under Methanogenic Conditions, 49 Applied Environ. Microbiol. 1080-1083 (1985), demonstrated that DCE and VC were generated during biodegradation of PCE under anaerobic conditions. Finally, Freedman and Gossett, *supra*, reported complete dechlorination of PCE to ethylene as follows: PCE → TCE → DCE → VC → ethylene. In each step of the process the compound was reduced (gaining two electrons) through substitution of a chlorine atom by a hydrogen atom. Hence this degradation pathway is often referred to as reductive dechlorination.

In the reductive dechlorination process, chloroethenes act as electron acceptors. This implies that the process can be limited in the field by the availability of sufficient suitable electron donors. In fact, reductive dechlorination also can be totally or partially inhibited by the

presence of competing inorganic electron acceptors, such as oxygen, nitrate, iron, and sulfate. It is now widely accepted that reductive dechlorination occurs to some extent at most field sites where chloroethene contamination exists in the presence of a sufficient supply of electron donors (P.L. McCarty, Biotic and Abiotic Transformations of Chlorinated Solvents in Groundwater, in Symposium on Natural Attenuation of Chlorinated Organics in Ground Water 5-9 (Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C., EPA/540/R-96/509, 1996); J.M. Gossett & S.H. Zinder, Microbiological Aspects Relevant to Natural Attenuation of Chlorinated Ethenes, in Symposium on Natural Attenuation of Chlorinated Organics in Ground Water 10-13 (Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C., EPA/540/R-96/509, 1996); T.H. Wiedemeier et al., Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Groundwater, Draft - Revision 1 (Air Force Center for Environmental Excellence, Technology Transfer Division, Brooks Air Force Base, San Antonio, Texas, 1997).

Many oxidizable organic compounds potentially could make suitable electron donors. For a potential electron donor to be useful as an amendment for enhanced *in situ* bioremediation, however, it must be safe to use, facilitate the desired reaction, and be relatively inexpensive. Lactate is a potential electron donor having these properties. It is innocuous enough for use in the food and medical industries. It has been demonstrated to facilitate reductive dechlorination of chlorinated solvents in several laboratory studies (e.g., W.P. DeBruin et al., Complete Biological Reductive Transformation of Tetrachloroethylene to Ethane, 58 Applied Environ. Microbiol. 1996-2000 (1992); S.A. Gibson & G.W. Sewell, Stimulation of Reductive Dechlorination of Tetrachloroethene in Anaerobic Aquifer Microcosms by Addition of Short-

Chain Organic Acids or Alcohols 1392-1393 (1992), D.E. Fennel et al., Comparison of Butyric Acid, Ethanol, Lactic Acid, and Propionic Acid as Hydrogen Donors for Reductive Dechlorination of Tetrachloroethene, 31 Environ. Sci. Technol. 918-926 (1997). The cost-effectiveness of lactate has not been thoroughly evaluated, but preliminary testing suggests that it will be at least as cost-effective as other *in situ* remediation technologies.

While the use of lactate as an electron donor to facilitate reductive dechlorination is well-established, it has only been applied for remediation of aqueous-phase contaminants because of the perception that bioremediation does not significantly enhance mass transfer of contaminants from the nonaqueous phase. It is shown herein, however, that the addition of high concentrations of a lactate solution not only facilitates reductive dechlorination of aqueous chloroethenes, but also significantly enhances mass transfer of nonaqueous contaminants, making them highly bioavailable. As used herein, "high concentrations" means high relative to the stoichiometric requirement for electron donor to degrade TCE to ethene. Thus, "high concentrations" means about 3-5 orders of magnitude greater than such stoichiometric requirements.

Facilitated transport and enhanced bioavailability of nonaqueous chlorinated solvents through addition of high concentrations of an appropriate electron donor, according to the present invention, take advantage of the natural processes that have made natural attenuation so popular, while also significantly reducing source longevity by enhancing mass transfer to the aqueous phase. The capital costs of the approach are minimal, because only a simple, potentially portable, injection system and monitoring wells are required. Initial mass removal may be slower than some of the other technologies, but it is sustainable for a relatively low cost and requires no extraction of contaminated ground water except for routine monitoring.

High concentrations of lactate, for example, not only provide an electron donor to expedite reductive dechlorination, but also facilitate mass transfer of the nonaqueous chlorinated solvents into the aqueous phase in a manner that makes them highly bioavailable. The lactate appears to act as a surfactant or co-solvent that brings nonaqueous chlorinated solvents into solution. The intimate contact of the chlorinated solvents (electron acceptors) in solution with the lactate (electron donor) enhances bioavailability and leads to rapid biodegradation. The depletion of the residual contamination source is potentially greatly accelerated due to the surfactant or co-solvent effect. The use of lactate to facilitate transport of chlorinated solvents into the aqueous phase and dramatically increase their bioavailability opens up a wide range of applications for enhanced *in situ* bioremediation of chlorinated solvents present as nonaqueous phase liquids at residual saturation in ground water. The use of a relatively inexpensive compound that accomplishes the same thing as mild surfactants or co-solvents, but does not require extraction and above-ground treatment, combines the advantages of mass removal with those of enhanced bioremediation.

All of the advantages of bioremediation, such as low capital cost, *in situ* contaminant destruction, unobtrusive appearance, public acceptance, low maintenance requirements, and the like, can be applied to residual source areas because, using this process, source longevity can potentially be greatly reduced. Many of these benefits are enjoyed by owners of contaminated sites, but reduced risk of further releases of contaminants to the public and the environment is also important.

The most appropriate application of this process is to sites with residual chlorinated solvent source areas in the subsurface, comprising primarily nonaqueous contaminants at residual

saturation. These are common at both federal and industrial facilities. When very large, mobile DNAPL pools are present, mass transfer rates may be too slow to effect remediation in a reasonable time frame, and more aggressive, capital-intensive approaches may be warranted.

Example 1

A 1-year field evaluation of enhanced *in situ* bioremediation was performed at Test Area North ("TAN") of the Idaho National Engineering and Environmental Laboratory. FIG. 1 shows a site plan of TAN, wherein solid symbols represent monitoring wells (10) and open symbols represent injection wells (12). The locations of a 5,000 μg/L TCE isopleth (14); 1,000 μg/L TCE isopleth (16); 100 μg/L TCE isopleth (18); and 5 μg/L TCE isopleth (20) are shown by solid lines. FIG. 2 illustrates a cross section of this site, showing the surface of the ground (22), an approximately 63-m (210-feet) fractured basalt unsaturation zone (24) (not drawn to scale), an approximately 60-m (200-feet) fractured basalt aquifer (26), and an impermeable clay interbed (28). The approximate location of the TCE secondary source (30) and the 1,000 μg/L TCE isopleth (32) are also indicated. The test was performed to determine whether this technology has the potential to enhance or replace the default pump-and-treat remedy selected for the contaminant source area in the site's Record of Decision. The residual source of chloroethenes (30), primarily TCE with some PCE and DCE, is present in the fractured basalt aquifer at the site, about 60 to 120 m below land surface. The residual source area (30) is approximately 60 m in diameter, and the TCE plume emanating from the this source is approximately 3 km long. Based on results of published studies and site-specific laboratory studies (K.S. Sorenson, Design of a Field-Scale Enhanced In Situ Bioremediation Evaluation for Trichloroethene in Ground

Water at the Idaho National Engineering and Environmental Laboratory, ASAE, St. Joseph, Michigan, Paper No. PNW98-113 (1998)), sodium lactate was chosen as the electron donor and was injected in Well TSF-05 in concentrations ranging from 3% to 60% by weight (Table 2).

The initial electron donor addition strategy involved continuous injection of potable water at 37.85 liters/minute (10 gpm) into Well TSF-05. The electron donor was to be pulsed into this line biweekly. The potable water injection began on November 16, 1998, at the beginning of the startup sampling period. Potable water injection was discontinued on December 11, 1998, due to a significant depression of chlorinated ethene concentrations near the injection well. It was determined that the continuous injection of clean water at 37.85 liters/minute (10 gpm) overwhelmed the flux of contaminants from the secondary source. This condition was considered undesirable for evaluation of an *in situ* technology, so the electron donor addition strategy was modified such that potable water was only injected for 1 hour following injection of the electron donor solution to flush the solution into the formation surrounding the injection well. This was intended to prevent significant quantities of electron donor from collecting in the injection well and to help prevent biofouling.

The raw electron donor solution used was food grade sodium lactate. Table 2 presents the injection date, the sodium lactate concentration in percent by weight, the injection rate in units of gallons per minute, the total volume of electron donor injected in gallons, and the volume in gallons of potable water injected at 75.7 liters/minute (20 gpm) to flush the solution into the formation. Lactate injections began on January 7, 1999, and were continued until September 8, 1999. Four injection solution concentrations were used, each being more dilute than the previous solution. The dilutions were made in an effort to keep the lactate in the upper

part of the aquifer, reducing density effects that cause the electron donor solution to sink to the base of the aquifer. Because the total mass of lactate was kept constant, and the injection flow rate was not dramatically increased, the duration of injection increased from 30 minutes to 4 hours.

Table 2

Date	Sodium Lactate Concentration (%)	Injection Flow Rate (gpm)	Total Volume Injected (gal)	Potable Water Flush Volume (gal)
1/7/1999	60	10	300	1,200
1/12/1999	60	10	300	1,200
1/19/1999	60	10	300	1,200
2/2/1999	30	20	600	1,200
2/9/1999	30	20	600	1,200
2/16/1999	30	20	600	1,200
2/23/1999	30	20	600	1,200
3/2/1999	6	25	1,500	1,200
3/4/1999	6	25	1,500	1,200
3/9/1999	6	25	1,500	1,200
3/11/1999	6	25	1,500	1,200
3/16/1999	6	25	1,500	1,200
3/18/1999	6	25	1,500	1,200
3/23/1999	6	25	1,500	1,200
3/25/1999	6	25	1,500	1,200
3/30/1999	6	25	1,500	1,200
4/1/1999	6	25	1,500	1,200
4/6/1999	6	25	1,500	1,200

4/8/1999	6	25	1,500	1,200
4/13/1999	6	25	1,500	1,200
4/15/1999	6	25	1,500	1,200
4/22/1999	6	25	3,000	1,200
4/28/1999	6	25	3,000	1,200
5/5/1999	6	25	3,000	1,200
5/12/1999	6	25	3,000	1,200
5/19/1999	6	25	3,000	1,200
5/26/1999	6	25	3,000	1,200
6/2/1999	6	25	3,000	1,200
6/9/1999	3	25	6,000	1,200
6/16/1999	3	25	6,000	1,200
6/23/1999	3	25	6,000	1,200
6/30/1999	3	25	6,000	1,200
7/7/1999	3	25	6,000	1,200
7/14/1999	3	25	6,000	1,200
7/21/1999	3	25	6,000	1,200
7/28/1999	3	25	6,000	1,200
8/4/1999	3	25	6,000	1,200
8/11/1999	3	25	6,000	1,200
8/18/1999	3	25	6,000	1,200
8/25/1999	3	25	6,000	1,200
9/1/1999	3	25	6,000	1,200
9/8/1999	3	25	6,000	1,200

Eleven monitoring wells (i.e., TAN-D2, TAN-9, TAN-10A, TAN-25, TAN-26, TAN-27,

TAN-28, TAN-29, TAN-30A, TAN-31, and TAN-37) were sampled biweekly and analyzed for electron donors, biological activity indicators, competing inorganic electron acceptors and their reduced products, chloroethenes, ethene, pH, temperature, and specific conductivity.

Electron Donor Distribution. Because concentrated lactate solutions are denser than water, their injection into an aquifer can cause density-driven flow downward in the aquifer. At TAN, some density-driven flow was desirable during lactate addition because the zone to be treated was approximately 60 m thick but the injection well (TSF-05) was completed only in the upper 30 m. It was apparent after the first month of injections, however, that too much of the lactate solution was moving into the lower half of the zone before spreading horizontally in the upper half of the zone. For this reason, the concentration of the lactate was reduced and the injection duration was increased in steps over several months. The importance of the lactate addition strategy can be seen in well TAN-31, a cross-gradient well completed in the upper half of the treatment zone approximately 15 m from the injection well (FIG. 3A). The increasing lactate concentrations after 150 days correspond to the third (and final) step in changing the injection strategy.

Redox Conditions and Reductive Dechlorination. The effect of lactate addition on redox conditions, and ultimately on reductive dechlorination, is evident in FIGS. 3A-C. Sulfate reduction actually began at the fairly modest lactate concentrations in well TAN-31 during the first 100 days of the test, with minor iron reduction evident from increasing ferrous iron concentrations (FIG. 3B). After sulfate was depleted, TCE transformation to *cis*-1,2-dichloroethene (*cis*-DCE) began (FIG. 3C). Reductive dechlorination stopped at *cis*-DCE until the lactate concentrations increased after 150 days and methanogenesis began. Transformation of

cis-DCE to vinyl chloride and ethene coincided almost exactly with the onset of methanogenesis. Beyond about 200 days from the start of the test, ethene was by far the largest constituent at this sampling location.

Enhanced reductive dechlorination of TCE to ethene was observed in all wells receiving significant lactate concentrations.

Based on the results of the field evaluation, enhanced *in situ* bioremediation was selected to replace pump-and-treat for remediation of the residual contaminant source area at Test Area North. Of particular importance in the decision process was the fact that the process was effective not only for degrading chlorinated solvents in the aqueous phase, but also that the process seemed to have a significant impact on the residual source itself.

Enhanced Bioavailability. A surprising observation during the field evaluation was a dramatic increase in TCE concentrations deep in the aquifer soon after sodium lactate addition began (FIG. 4). The TCE increase appeared to occur essentially simultaneously with the arrival of the highly concentrated electron donor solution. In addition, the peak TCE concentration was actually significantly higher than historical measurements for well TAN-26. These observations strongly suggest that the transport of TCE to well TAN-26 was associated with the downward migration of the electron donor. This could occur through two mechanisms. One possible explanation for the large, rapid increase in TCE concentrations is that the lactate solution simply pushed secondary source material along in front of it as it migrated out from well TAN-05, through the secondary source, and down toward well TAN-26. However, tritium was a co-contaminant in the residual source material, and consideration of the tritium data in well TAN-26 appears to rule out this possibility. In fact, tritium concentrations were completely unaffected in

spite of large increases in organic contaminant concentrations (TCE and DCE).

A second possible explanation for increased TCE concentrations in well TAN-26 is that the lactate injection led to facilitated transport of the organic contaminants. Three hypotheses that could explain facilitated transport are as follows: (1) that the lactate solution acts as a co-solvent for the organic contaminants, (2) that the lactate acts as a surfactant, and (3) that the lactate solution, because of its high concentration, displaces sorbed chlorinated ethenes, driving them into solution. All of these mechanisms would result in facilitated transport of the chlorinated ethenes in intimate contact with the lactate solution and would make more of the chlorinated ethenes bioavailable. The behavior of the TCE in well TAN-26 after the peak concentration suggests that it was, in fact, extremely bioavailable. The drop in TCE concentration from the peak concentration to undetectable levels occurred with a TCE half-life of less than 20 days (assuming first-order kinetics for illustration). Just as important for the facilitated transport hypothesis, *cis*-DCE increased to a peak concentration within 20% of the peak TCE concentration (indicating an excellent mass balance), and then remained elevated near that peak concentration. The significance of this point is that the lactate injection was continuing, so if the hypothesis were valid it would be expected to continue bringing the organic contaminants with it as it migrated through the secondary source. After biological activity increased, the TCE was transformed to *cis*-DCE before reaching well TAN-26, but as shown in FIG. 4, the total ethene level remained approximately constant. After several months the total ethene concentration dropped, but this was expected (and intentional) because the lactate solution concentration had been reduced by a factor of 20 in June. This change reduced the density of the solution significantly, so less lactate, and therefore less total ethenes, was transported to well

TAN-26. Thus, the concentration decrease supports the hypothesis of facilitated transport.

The facilitated transport makes available for reductive dechlorination large quantities of the chlorinated ethenes that otherwise would remain associated with the secondary source. As shown by the well TAN-26 data, once made available by the lactate solution, the TCE was, in fact, rapidly degraded. Enhanced bioavailability of chlorinated ethenes in the secondary source would greatly decrease the longevity of the source.

Example 2

Based on the field results presented in Example 1, laboratory studies were preformed to confirm that the enhanced bioavailability of TCE observed in the field was due to co-solvent or surfactant behavior resulting from the use of high concentrations of sodium lactate. Two fundamental properties used to screen the co-solvent or surfactant properties of a solution are surface tension and interfacial tension. Surface tension measures the force per unit length along the interface between a liquid and air due to its tension. When a co-solvent or surfactant is present in an aqueous liquid at increasing concentrations, the surface tension of that liquid decreases. Interfacial tension is similar to surface tension except that it measures the force per unit length along the interface between two liquid phases arising from the surface free energy. The higher the interfacial tension between two liquids, the less likely one is to dissolve into the other, and the more difficult it is for one to be transported within the other. Thus, perhaps the most significant property of co-solvents and surfactants in the context of chlorinated solvent remediation is that they decrease the interfacial tension between the aqueous phase (groundwater) and the organic nonaqueous phase so that the solubility (or mobility for order-of-magnitude

decreases) of the nonaqueous phase is enhanced.

The laboratory study performed to confirm the co-solvent properties of the high concentration electron donor solution measured the surface tension of electron donor solutions at various concentrations. Next, interfacial tensions between the same electron donor solutions and nonaqueous phase TCE were measured. Two types of electron donor solutions were used. The first was different concentrations of sodium lactate, the electron donor used in Example 1. The second was various mixtures of sodium lactate and ethyl lactate. Ethyl lactate was chosen because it is a lactate-based compound that is used in some industries as a solvent. Thus it was believed ethyl lactate might further enhance the co-solvent behavior observed, while still acting as a suitable electron donor for bioremediation. It is believed that mixtures of sodium lactate and ethyl lactate have never before been used for bioremediation. Surface and interfacial tension measurements were made using the pendant drop method (M.J. Rosen, ed., Structure/Performance Relationships in Surfactants, American Chemical Society, Washington D.C. 329 (1984); R.D. Bagnall & P.A. Arundel, The Profile Area of Pendant Drops, 82 J. Phys. Chem. 898 (1978)) coupled with real-time video imaging (M.D. Herd et al., Interfacial Tensions of Microbial Surfactants Determined by Real-Time Video Imaging of Pendant Drops, Proceedings paper number SPE/DOE 24206 513-519, SPE/DOE Eighth Symposium on Enhanced Oil Recovery, Tulsa, OK (1992)).

The results of the surface tension experiment are shown in Figure 5. Surface tension is plotted on the vertical axis, while sodium lactate concentration for each solution is plotted on the horizontal axis. The different lines on the plot are for different concentrations of ethyl lactate ranging from 0 to 10% mixed with the sodium lactate solution. Error bars represent two standard

deviations around the mean. Examination of the 0% ethyl lactate line (sodium lactate only) reveals that at sodium lactate concentrations from 0.01 to 7%, almost no change in surface tension occurred. As the concentration was increased to 30 and 60%, however, a dramatic decrease in the surface tension was measured. This result confirms that sodium lactate begins to exhibit co-solvent properties at high concentrations. These concentrations are about 3 orders of magnitude higher than reported in other studies, which explains the surprising results discussed in Example 1.

In an effort to decrease the sodium lactate concentrations required to lower the surface tension of the solution, mixtures with ethyl lactate were evaluated. As seen in Figure 5, the addition of 1% and 10% ethyl lactate to the different sodium lactate solutions had a pronounced effect on the solution's surface tension. Thus, the addition of ethyl lactate to the sodium lactate electron donor solution enhances its co-solvent properties. The choice of optimum concentration would be a matter of design for a specific remediation. If only slightly enhanced bioavailability of the solvents were desired, the high concentration sodium lactate solution would be appropriate. If a large degree of enhanced bioavailability were desired, the addition of 1 to 10% ethyl lactate would be appropriate.

The results of the interfacial tension measurements are shown in Figure 6. As before, error bars represent two standard deviations around the mean. For 0% ethyl lactate (sodium lactate only), the effect of increasing sodium lactate concentration occurs at lower concentrations for interfacial tension than observed in the surface tension measurements. Interfacial tension decreased by about 26% when sodium lactate was increased from 0.1 to 3% (still 2 orders of magnitude above previous studies). When sodium lactate was increased to 30%, the interfacial

tension was decreased to 47% of the value at a sodium lactate concentration of 0.1%. Again, the importance of high sodium lactate concentrations for achieving the co-solvent properties is apparent.

As ethyl lactate was added to the sodium lactate solutions, it is clear that the ethyl lactate concentration is the primary factor affecting interfacial tension. Figure 6 shows that the interfacial tension becomes relatively insensitive to sodium lactate concentration for the ethyl lactate mixtures. From a remediation design standpoint, this simplifies things because co-solvent effects appear to be affected by only one component of the mixture. Interestingly, only the 10% ethyl lactate mixture displayed lower surface tensions than the 30% sodium lactate solution with no ethyl lactate.

Example 3

Development of Electron Donors for Chlorinated Solvent Source Area Bioremediation

1. THE PROBLEM AND THE OPPORTUNITY

Chlorinated solvents are the most common class of contaminants in ground water at hazardous waste sites in the U.S. In 1993, the Agency for Toxic Substances and Disease Registry (ATSDR) compiled a list of the top 25 contaminants detected at hazardous waste sites on the National Priorities List (NPL). The ATSDR ranking identified eight of the top 20 contaminants as chlorinated solvents and their intrinsic degradation products, including two of the top three (4). The ranking was updated by the ATSDR on their internet site based on 1996 data with similar results. Of particular significance is the identification of trichloroethene (TCE) and tetrachloroethene (PCE) as the first and third most common contaminants at NPL sites in both surveys. Not surprisingly chlorinated solvents are also the most common contaminants at Department of Defense sites and are present at virtually every Department of Energy facility.

The prevalence of chlorinated solvents is due both to their widespread use and to their longevity in the environment. Their longevity is partly due to the hydrophobic nature that makes them such good solvents, as well as their relatively oxidized states that prevent them from serving as electron donors for microorganisms. At many sites, the subsurface solvent sources referred to as dense nonaqueous-phase liquids (DNAPLs) are present. DNAPLs are hydrophobic liquids with a density greater than water. Pertinent to their longevity is the fact that the solubility of the common chlorinated solvents (PCE, TCE, TCA, and carbon tetrachloride) ranges from about 200 to 1,400 mg/L at 25°C (5). These relatively low solubilities play a significant role in

limiting mass transfer to the aqueous phase once the solvents contaminate ground water. Interphase mass transfer (dissolution) of a solvent NAPL into ground water is governed by the difference between the aqueous solubility of the compound and the actual concentration in ground water (Sale (6) provides an excellent discussion of fundamental interphase mass transfer from DNAPLs). At typical ground water velocities, the aqueous concentration of the solvent in the immediate vicinity of the ground water-NAPL interface approaches the solubility within the first few centimeters of flow along the interface (6). Because ground water flow is generally laminar, very little mixing of the water near the interface occurs with water even a few centimeters from the interface.

The lack of mixing characteristic of laminar flow has at least two important implications. First, it explains why ground water concentrations of chlorinated solvents greater than 10% of their solubility are rarely measured, even at contaminated sites with large quantities of DNAPL. Second, the attainment of concentrations approaching solubility within a few centimeters of ground water flow along the interface effectively prevents mass transfer out of the DNAPL for the remainder of flow along the interface. For example, if ground water flows across a pool of DNAPL (or through an area of residual saturation) several meters long in the direction of flow, mass transfer into the aqueous phase will be insignificant along all but the first few centimeters of the flow path. The result is that chlorinated solvents persist in ground water for many decades, or perhaps even centuries.

Cleanup of chlorinated solvent sources in ground water is often considered technically (or economically) impracticable because of their density and hydrophobicity, often compounded by subsurface heterogeneity. As a result, many sites have resorted to pump and treat or other containment technologies. Operations and maintenance costs of such systems become very large

over time, however, because of the longevity of the subsurface sources discussed above. As noted in the description of Topic Area E for this proposal, "in-situ technologies that mobilize contaminants to make them more amenable to subsequent in-situ treatment" are needed. It was also noted that "Special needs include better methods for locating DNAPL pockets and cost-effective destruction technologies."

While significant progress has been made in addressing DNAPL source areas, parties responsible for cleaning up sites with chlorinated solvent residual source areas in ground water are still faced with several technologies with significant capital costs, secondary waste streams, the involvement of hazardous materials or energy, and the potential for additional worker or environmental exposure. A more ideal technology would involve lower capital costs, would not generate secondary waste streams, would be non-hazardous to workers and the environment, would destroy contaminants in situ, would be low maintenance, and would minimize disturbance of the site. Bioremediation satisfies all of the characteristics of an ideal technology listed above; however, it has traditionally been viewed as very passive with respect to source area remediation. That is, conventional wisdom suggests that bioremediation is limited by the rate at which nonaqueous contaminants dissolve or diffuse to where bacteria can degrade them. If that were true, bioremediation would still have all the benefits of an in situ technology regarding low capital cost, lack of secondary waste streams, low maintenance, minimal site disturbance, etc., but would not be much different than pump-and-treat in terms of cleanup times. Recent advances have shown however, that mass transfer rates of chlorinated solvents from the nonaqueous phase to the aqueous phase (where they are bioavailable) can be substantially increased during bioremediation. In fact, North Wind Environmental, Inc. is currently licensed to implement the patent-pending Bioavailability Enhancement Technology™, developed by the

proposed principle investigator while working at the Department of Energy's Idaho National Engineering and Environmental Laboratory. This technology takes advantage of the ability of certain electron donor solutions to enhance mass transfer of DNAPLs, while stimulating in-situ bioremediation.

While the technology has been demonstrated with conventional electron donors, preliminary work with novel electron donors and electron donor mixtures shows the potential to improve performance of the technology significantly. The goal of this proposal is to advance the development of a suite of novel electron donors that will propel Bioavailability Enhancement Technology™ to the forefront of the DNAPL remediation market because of the technical, cost, human, and environmental benefits relative to existing technologies. It is important to note that this is ***not*** simply a proposal to evaluate yet another host of electron donors to stimulate reductive dechlorination of chlorinated solvents, but that it builds upon a paradigm shift in the understanding of DNAPL bioremediation by developing electron donors with very special properties to accelerate mass transfer, mobilizing the contaminants for subsequent in-situ destruction.

To fully understand the benefits of the extension of bioremediation to DNAPL source zone remediation, one must look at all aspects of its implementation in comparison to currently available technologies. Conventional technologies for cleaning up residual source areas (sites with residual nonaqueous phase solvents) in ground water typically involve high capital costs and generate significant secondary waste streams. Table 1 summarizes technologies used for this application. Pump and treat is included because it is often used as the baseline technology for ground water remediation and is used for containment of chlorinated solvent source areas, although cleanup timeframes for pump and treat range from decades to centuries. Aerobic and

dissolved phase anaerobic bioremediation are included to distinguish them from Bioavailability Enhancement Technology™.

Table 1. Comparison of Remediation Technologies for Chlorinated Solvent Source Areas.

	Capital Cost	Operating Cost	Waste Mgmt. Cost	Potential Worker or Environmental Exposure?	Contaminant Transfer or Destruction?	Applicable to Source Areas?	Applicable to Large NAPL Volumes?	Applicable to Dissolved Phase?	Potentially Hazardous Materials or Energy?	Risk of Detrimental Source Mobilization?
Pump and Treat	Medium	Low to Medium	Low to Medium	Yes	Transfer	No (except containment)	No	Yes	No	No
Thermal (Steam or Electrical)	High	High	Medium to High	Yes	Both	Yes	Yes	No	Yes	Yes
Chemical Oxidation	Low to Medium	Low to Medium	Low	No	Destruction	Yes	Yes	No	Yes	No
Surfactant or Cosolvent Flooding	Medium to High	Medium	High	Yes	Transfer	Yes	Yes	No	No	Yes
Aerobic Bioremediation	Medium	Low to Medium	Low	No	Destruction	No	No	Yes	Yes	No
Dissolved Phase Anaerobic Bioremediation	Low to Medium	Low to Medium	Low	No	Destruction	No	No	Yes	No	No
Anaerobic Bioremediation with Bioavailability Enhancement Technology™	**Low to Medium**	**Low to Medium**	**Low**	**No**	**Destruction**	**Yes**	**?**	**Yes**	**No**	**No**

From the table it is clear that parties responsible for cleaning up sites with chlorinated solvent residual source areas in ground water are faced with several technologies with significant capital costs, secondary waste streams, the involvement of hazardous materials or energy, and

the potential for additional worker or environmental exposure. A more ideal technology would involve lower capital costs, would not generate secondary waste streams, would be non-hazardous, would destroy contaminants in-situ, would be low maintenance, and would minimize disturbance of the site. Bioremediation satisfies all of these desires, and with the current and proposed advancements showing that biological processes can be used that enhance the bioavailability of nonaqueous contaminants, this technology is now applicable to residual source areas where it was previously thought to be ineffective. That is, Bioavailability Enhancement Technology™ provides the benefits of aggressive, expensive, and even dangerous technologies with those of a passive, inexpensive, in-situ technology. The further development of novel electron donors has the potential both to increase the former benefits by improving enhanced mass transfer characteristics and to increase the latter benefits by improving longevity and transportability. All of these advancements will dramatically reduce the cost of DNAPL remediation.

2. PHASE I OBJECTIVES

Researchers and practitioners are trying a variety of electron donors in the laboratory and in the field to stimulate reductive dechlorination of chlorinated solvents. They are selected based on cost, demonstrated effectiveness, solubility, transport properties, longevity, and other factors. For effective bioremediation of source areas, recent research (Section 4) indicates they should also be selected based on their impact on contaminant dissolution rates. Ideally an electron donor would exhibit maximum contaminant degradation, longevity, transportability, and dissolution enhancement, along with minimum cost. While it is unlikely that a single electron donor would fit this description for all sites, our preliminary work suggests that a small suite of electron donors would cover the range of needs for most sites. The primary objective of this Phase I proposal is to develop such a suite of electron donors that improves upon compounds already in use either by enhancing their existing desirable properties, or by adding new properties to increase their effectiveness and decrease the cost of application. The specific technical objectives for Phase I are listed below:

1. Evaluate the transport properties of a suite of novel electron donors and electron donor mixtures and compare to conventional electron donors. The more easily an electron donor can be transported in the subsurface, the lower the cost to distribute it throughout a large volume.

2. Evaluate the extent to which the suite of electron donors enhances the dissolution of PCE and TCE from the nonaqueous phase. The greater the dissolution enhancement, the faster the cleanup, and the lower the cost.

3. Evaluate the utility of the suite of electron donors for enhancing reductive dechlorination based on the fermentation products generated. A variety of organic acids have been documented to stimulate reductive dechlorination reliably, and their generation indicates a high probability a given electron donor will be effective.

4. Evaluate the longevity of the suite of electron donors. The longer a given electron donor continues to provide organic acids and hydrogen for reductive dechlorination, the less frequently applications are required, and the lower the cost.

Meeting these Phase I objectives will result in the selection of a small suite of electron donors, or perhaps even a single electron donor solution, that will exhibit an optimal range of characteristics for facilitating bioremediation of chlorinated solvent source areas. In particular, the electron donor(s) will significantly enhance dissolution of nonaqueous phase chlorinated solvents through Bioavailability Enhancement Technology™, will be easily transported so large areas can be treated cost effectively, will provide appropriate fermentation products to stimulate reductive dechlorination, and will last long enough to minimize the frequency of reapplication. Having accomplished this, the stage will be set for a Phase II effort in which the electron donor(s) can be tested at field scale (Section 6).

3. PHASE I WORK PLAN

The Phase I objectives will be met through a two-pronged approach. The first component of the work is a series of laboratory column studies. The columns will be used to evaluate the transport properties of various electron donors through a porous medium (Objective 1). They will also be used to evaluate the degree of enhanced dissolution of PCE and TCE achieved by the electron donors (Objective 2). The second component of Phase I is a set of microcosm studies.

The microcosms will be inoculated with soil and/or groundwater free of contaminants, and spiked with each of the electron donors. The microcosms will then be used to evaluate the utility of the electron donors for enhancing reductive dechlorination based on the fermentation products produced (Objective 3), as well as measuring their longevity (Objective 4). The two components of Phase I will be performed in parallel. A detailed description of the work follows.

3.1 Column Studies

The column studies will be based on a design used by Brennan et al. (in preparation), that was used to demonstrate enhanced dissolution of PCE DNAPL due to the influence of chitin fermentation products. Their design was partially based on that of Wild and Reinhard (37). Several columns will be set up vertically, packed with quartz sand, then flushed upward with degassed, deionized water to remove trace fines and entrapped air. The columns will then be loaded with PCE or TCE DNAPL, and flushed downward with water to achieve residual saturation. Retention times for the columns at a given DNAPL residual saturation will be verified through conservative tracer tests using bromide.

Once the columns are prepared and retention times established, the enhanced dissolution experiments will begin. Several electron donor solutions will be tested that, based on preliminary testing discussed in Section 4, are likely to significantly enhance dissolution rates of DNAPL. Some of the compounds that are likely to be included either individually or in mixtures are sodium lactate, ethyl lactate, oleyl lactyllic acid, whey derivatives, and vegetable oil emulsions **(confidential proprietary information)**. The use of vegetable oil emulsions will serve to provide a comparison of the novel electron donor solutions to a solution currently being

used in the field. In addition to the columns flushed with electron donor solutions, an identical column will be flushed with unamended water as a negative control.

The duration for each column experiment will depend on the performance of the electron donor solution compared to unamended water with respect to enhanced dissolution. It is planned to perform two sets of column studies during Phase I to test a variety of electron donor solutions (see schedule in Section 3.4). Samples will be collected from the column effluent daily at first, but the frequency may change depending on the rate of change of concentrations. The samples will be analyzed for both the DNAPL (PCE or TCE) and total organic carbon (TOC). Each of the electron donor stock solutions will be analyzed in triplicate for TOC to establish a ratio to electron donor concentration. In-line pressure transducers will be used to monitor clogging of the column over time that may occur with higher viscosity electron donors.

The data collected from the column studies will be used for the first two technical objectives of the Phase I work. The TOC data, together with the pressure monitoring will provide the information necessary to assess the relative transportability of the various electron donors. These data will also be compared to the transport of the conservative tracer through the columns. The PCE or TCE measurements will allow determination of the extent to which dissolution of the DNAPL is enhanced by the different electron donor solutions as compared to flushing with unamended water.

3.2 Microcosm Studies

Microcosms will be set up using standard methods (e.g., 38). Serum bottles will be used, most likely with Teflon^{TM}-lined butyl rubber stoppers and aluminum crimp caps. The bottles will be stored upside down, in the dark, at 20°C throughout the study. The microcosms will be

loaded with soil and or groundwater from an uncontaminated location to provide an environmental consortium as the inoculum. They will then be amended with an electron donor solution and monitored.

Each electron donor solution tested will be set up in triplicate. At a minimum, the same solutions tested in the column experiments will be used, but a broader range of mixtures may be evaluated in the microcosms because of the ease of setup. Each microcosm will be sampled weekly for the duration of the study. The samples will be analyzed for volatile fatty acids, TOC, dissolved inorganic carbon, and methane.

The data collected from the microcosms will be used for the third and fourth objectives of the Phase I work. Monitoring volatile fatty acids will demonstrate whether fermentation of a given electron donor solution produces appropriate compounds at appropriate concentrations to facilitate reductive dechlorination. Those data, along with TOC, dissolved inorganic carbon, and methane, will be used to estimate fermentation rates and relative longevity of the various electron donor solutions. As mentioned above, the electron donor stock solutions will be analyzed for TOC to establish the ratio of TOC to electron donor concentration.

3.3 Analytical Methods

Volatile organic compounds will be measured with an HP 5890 gas chromatograph utilizing a flame ionization detector (e.g., 38). Methane will likewise be analyzed by gas chromatograph with flame ionization detector (e.g., Brennan et al. in press). A high sensitivity, aqueous total organic carbon analyzer will be used to measure TOC as well as dissolved inorganic carbon. Volatile fatty acids will either be measured via high-performance liquid chromatography (e.g., Brennan et al. in press) or for some compounds using a gas

chromatograph with flame ionization detector (22). A modular ion chromatographic system (Dionex DX-320) will also be available that can be configured for analysis of volatile fatty acids.

4. RELATED RESEARCH AND DEVELOPMENT

Microbial reductive dechlorination of chlorinated ethenes has been well documented (1-11) and anaerobic bioremediation is currently being employed to treat chlorinated ethene contaminated groundwater (12); however, dechlorination-based source-zone restoration has not been rigorously evaluated. Investigations into dechlorination-based source zone restoration have begun only recently (15-17). Past efforts of source-zone remediation have been concerned with the potential toxicity of high contaminant concentrations on the microbial populations, but several studies have confirmed biological dechlorination occurring at aqueous saturation PCE concentrations and high concentrations of TCE (17, 11-14). Halorespiring organisms might actually have an advantage in environments of high PCE and TCE concentrations, such as NAPL source zones, where organisms normally competing with them for energy sources are not able to thrive (17, 18-21).

Most importantly, recent research has demonstrated that rapid rates of biological dechlorination in nonaqueous phase liquid (NAPL)-containing source areas can dramatically reduce the length of time that a NAPL will continue to be a source of chlorinated solvent contamination (15-17). Mechanisms that contribute to accelerated NAPL removal were discussed in Sorenson (in press). A combination of two processes is responsible for this observation in laboratory tests. First, the dechlorinating bacteria are capable of living in close proximity to the NAPL/water interface. Thus their metabolic activity increases the driving force for mass transfer (i.e., the concentration gradient). Second, the metabolic products of

dechlorination are less hydrophobic than the parent compounds and they partition more extensively to the aqueous phase. In recent laboratory studies conducted in glass-bead columns (2), Cope and Hughes observed a 16-fold increase in PCE removal from a NAPL in biotic systems as compared to abiotic "washout". This result was similar to the observation of a 14-fold increase in PCE removal rates from a NAPL in a continuous-flow stirred-tank reactor (1). If similar results are obtained in field systems, a 100-year source of PCE would be present for only 6.25 years.

In addition to the two mechanisms observed in the laboratory, Sorenson (22) presents a third mechanism initially observed in the field at the Idaho National Engineering and Environmental Laboratory, then investigated further in the laboratory. The third mechanism is different than the first two in that it relies not only on the biological activity stimulated by the electron donor, but is related to the physicochemical interaction of the electron donor solution and DNAPL. As shown in Figure 7, this resulted in significant enhanced mass transfer that caused a large spike in TCE concentrations. The newly dissolved TCE was eminently bioavailable, however, because it was mixed with the electron donor that ultimately led to its degradation, as shown by the stoichiometric conversion to cis-DCE. Subsequently, the cis-DCE was completely transformed to ethene. The mass balance was not completed for the ethene because the amount of lactate being transported to the monitoring location shown was reduced, thereby decreasing the contaminant "loading" (22). Behavior of stable carbon isotope ratios of TCE during the field test also suggested that the residual TCE DNAPL was being dissolved by the lactate injections (23).

Laboratory studies were performed to investigate the mechanism responsible for this dramatic increase of TCE concentrations induced by the sodium lactate injection, and it was found that high concentrations of sodium lactate decreased the interfacial tension between nonaqueous TCE and the electron donor solution by as much as 47% (Sorenson, in press). Decreasing interfacial tension between two liquids generally indicates an increasing effective solubility. Following this work, we began testing addition electron donor solutions and mixtures for their impacts on interfacial tension with TCE. Some of this work contributed to the patent application for Bioavailability Enhancement Technology™. The most recent studies show that a variety of electron donor solutions decrease interfacial tension when applied at high enough concentrations. This **confidential proprietary information** is shown in Figure 8.

The data points in the figure represent the mean interfacial tension measurement based on at least eight independent measurements. The error bars show one standard deviation. It can be seen that as electron donor concentration increases, interfacial tension generally decreases. While sodium lactate had dramatic effects in the field (see Figure 7), it appears that the other electron donor solutions tested might increase bioavailability to an even greater extent. Oleyl lactyllic acid is of particular interest from this group because it is a food-grade emulsifier that appears to combine a number of desirable effects of electron donors, as discussed further below.

As mentioned earlier, researchers and practitioners are trying a variety of electron donors in the laboratory and in the field to stimulate reductive dechlorination of chlorinated solvents. A significant portion of this work has focused on the concept of "slow-release" electron donors (24). That is, electron donors that are applied in a nonaqueous phase that dissolves slowly into surrounding groundwater. The primary benefit of slow-release electron donors is that they are

extremely low maintenance because renewed application is only required after several months or even a few years as long as sufficient electron donor is supplied to degrade the chlorinated solvents present in groundwater.

Slow-release electron donors that have been used in the field to date include vegetable oils (25-29), HRC® (30-34), bark mulch (35), and chitin (36; Brennan et al. in press) to name a few. While all of these electron donors have been shown to facilitate reductive dechlorination, they share some common disadvantages. First, because they are all nonaqueous, distributing them in the subsurface is typically difficult. The solid electron donors cannot be pushed through soils because they are filtered out. The oil-phase electron donors have high viscosities relative to water and are not easily transported below the water table. Finally the polymer, HRC®, suffers from both issues. The second disadvantage is that all of the slow-release electron donors, by definition, produce volatile fatty acids at low concentrations that have a much smaller effect on interfacial tension, and therefore bioavailability, than high concentration, dissolved electron donors such as sodium lactate. Thus, although the slow release electron donors last longer than other electron donors, they generally require more closely-spaced applications to cover a given area, and they do much less to shorten overall remediation timeframes.

A more ideal electron donor would have the longevity of a slow-release compound to minimize application frequency, but would be more easily transported so that fewer application points would be required to cover large areas, and would decrease interfacial tension significantly so that mass transfer and bioavailability would be enhanced and cleanup times shortened. Oleyl lactyllic acid may be just such an electron donor **(confidential proprietary information)**. It is essentially a combination of vegetable oil and lactic acid that was developed

in the food processing industry a few decades ago as an emulsifier. Our preliminary work with the compound illustrated in Figure 8 shows the power of the emulsification properties with respect to interfacial tension with TCE. Although it has an appearance and viscosity of vegetable oil, its nature as an emulsifier causes it to react very differently with water than pure vegetable oil. When vegetable oil is used in the field as electron donor, an emulsifier such as lecithin must be added to inject it into groundwater (25). The mixture is then mixed to achieve an emulsion of a few tenths of a percent to a few percent oil. The droplet size of the emulsion may be a critical factor in its transportability (26). In order to achieve small, easily transported droplets in the emulsion, intensive mixing is required. In contrast, oleyl lactyllic acid is itself an emulsifier and we have observed that it forms a stable microemulsion in water with very little mixing (unpublished data). This observation is consistent with its intended use in food processing. Thus, preliminary observations suggest oleyl lactyllic acid may have the qualities of transportability and enhanced bioavailability that are desired from aqueous electron donors such as lactate, while also having the slow release characteristics of vegetable oil. While this may be the most promising example on the surface, other potential electron donors will be evaluated under this proposal.

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